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13. ABSTRACT (Maximum 200 words) Progress has been made in two related areas during the period of this grant. In the area of luminescence-based biosensor systems, we have developed sensitive methods to detect antibiotics in biological materials and foods using bioluminescence. We believe that our methods can be automated and applied to the rapid detection of antibiotics in biological fluids, food sources, and other materials. In related studies, the crystal structure of bacterial luciferase has been determined at 2.4 Å resolution. This accomplishment represents a major step forward in our effort to understand this enzyme and how it works, including the details of its folding. Knowledge of the structure is crucial also to efforts to derivatize the enzyme for development of new generation biosensor systems.				
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FINAL TECHNICAL REPORT

OFFICE OF NAVAL RESEARCH

ONR Grant #

N00014-93-1-0991

Grant Title

**Luminescence-Based Biosensor
Systems**

Grant Period

September 1, 1993 - September 30, 1995

Principal Investigators

Thomas O. Baldwin and Miriam M. Ziegler

**Performing Organization:
The Texas Agricultural Experiment Station
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TAES ACCOUNT NUMBER: 500777

Accomplishments: Progress has been made in two related areas during the period of this grant. One area is in the development of luminescence-based biosensor systems, and the other is in the advancement of basic knowledge that will be crucial to long-term efforts to develop additional strategies for biosensor technologies.

We have developed sensitive methods to detect antibiotics in biological materials and foods using bioluminescence. The method takes advantage of the cell-density dependent (quorum sensing) system that controls the expression of bioluminescence. Exposure of cells preinduction to even low levels of antibiotics causes a slight attenuation of the induction process that is amplified as the culture grows, and is manifested by large differences in the intensity of light emission postinduction. We believe that our methods can be automated and applied to the rapid detection of antibiotics in biological fluids, food sources, and other materials.

In related studies, the high resolution crystal structure of bacterial luciferase has been determined (see refereed publication 11, below, and Figure 1). This accomplishment represents a major step forward in our effort to understand this enzyme and how it works, including the details of its folding. Knowledge of the structure is crucial also to efforts to derivatize the enzyme for development of new generation biosensor systems. While knowledge of the structure is an important milestone, utilization of the information will require much additional effort. We are making excellent progress in related studies under ONR grant #N0001496-1-0087, and we hope that future studies on this system will continue to be supported by the Office of Naval Research.

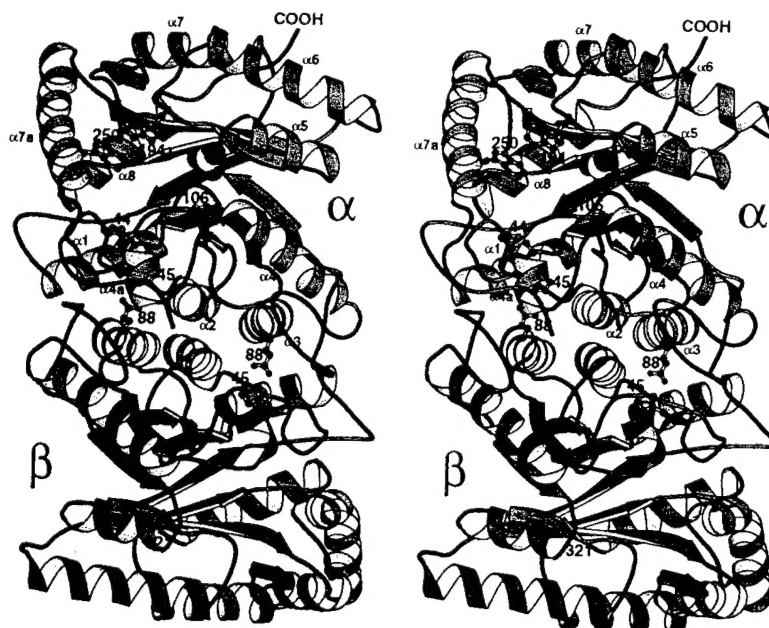


Figure 1. Stereo image of an α -carbon trace of the α and β subunits of bacterial luciferase from *Vibrio harveyi* (from the 2.4 Å structure, refereed publication #11 below).

Publications (1993-1995):

Refereed Publications

1. Devine, J. H., Kutuzova, G., Green, V. W., Ugarova, N. and Baldwin, T. O. (1993) Luciferase from the European firefly *Luciola mingrelica*: Cloning and nucleotide sequence of the cDNA, overexpression in *E. coli* and purification of the enzyme. *Biochim. Biophys. Acta* **1173**, 121-132.
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11. Fisher, A. J., Raushel, F. M., Baldwin, T. O. and Rayment, I. (1995) The three-dimensional structure of bacterial luciferase from *Vibrio harveyi* at 2.4 Å resolution. *Biochemistry* **34**, 6581-6586.

Book chapters, symposium contributions, reviews, etc

1. Francisco, W. A., Abu-Soud, H. M., Raushel, F. M. and Baldwin, T. O. (1993) Studies on the kinetic mechanism of the bacterial luciferase-catalyzed reaction. In Bioluminescence and Chemiluminescence, eds. A. A. Szalay, L. J. Kricka, and P. Stanley (John Wiley & Sons) pp. 113-117.
2. Ziegler, M. M., Clark, A. C., Sinclair, J. F., Chaffotte, A. F., Goldberg, M. E. and Baldwin, T. O. (1993) Folding and assembly of the subunits of bacterial luciferase. In Bioluminescence and Chemiluminescence, eds. A. A. Szalay, L. J. Kricka, and P. Stanley (John Wiley & Sons) pp. 178-182.
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